510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K222829

B Applicant

Meridian Bioscience Inc.

C Proprietary and Established Names

Curian Shiga Toxin

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
GMZ	Class I	21 CFR 866.3255 - Escherichia Coli Serological Reagents	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To determine substantial equivalence for the Curian Shiga Toxin assay used to detect Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) in cultures derived from stool samples.

B Measurand:

Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2)

C Type of Test:

Lateral flow fluorescent immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Curian Shiga Toxin assay, for use with the Curian Analyzer, is a rapid, qualitative, fluorescent immunoassay for the simultaneous detection and differentiation of Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) in a single test device. It is intended for use with cultures derived from human stool specimens to aid in the diagnosis of disease caused by Shiga toxin producing *Escherichia coli* (STEC) infections. Test results are to be used in conjunction with the patient's clinical symptoms and history.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

The Curian Shiga Toxin assay can only be run on the Curian Analyzer.

IV Device/System Characteristics:

A Device Description:

The Curian Shiga Toxin assay is a qualitative *in vitro* diagnostic test for the detection of Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) in cultures derived from human stool specimens. The Curian Shiga Toxin assay utilizes fluorescence technology with the cleared Curian Analyzer (K192817) to detect Stx1 and Stx2 in cultures derived from human stool.

The Curian Shiga Toxin assay kit contains the following components:

- Curian Shiga Toxin Test Card: A test strip enclosed in a plastic frame which is in a foil pouch with a desiccant. The desiccant will appear pink; if the packaging is compromised, the desiccant will be blue. Test steops are supplied ready to use.
- Curian Shiga Toxin Aioprep Sample Preparation Device/ Negative Control: A buffered aqueous protein solution containing blue dye and 0.094% sodium azide. The Aioprep device is fitted with a dropper tip. Test buffer is supplied ready to use.
- Curian Shiga Toxin Positive Control: Inactivated Shiga toxins 1 and 2 in an aqueous phosphate buffered solution containing 0.094% sodium azide. The positive control buffer is supplied ready to use.
- Pipette I: Transfer pipettes graduated to 50 µL and 175 µL.
- Pipette II: Transfer pipettes graduated up to 1.0 mL.

B Principle of Operation:

The Curian Shiga Toxin assay is a lateral flow-based fluorescent immunoassay for the direct detection of Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) in cultures derived from human stool specimens. The Curian Shiga Toxin assay is based on standard immunoassay sandwich technology utilizing two detector antibodies (*E. coli* monoclonal antibodies specific to Stx1 or Stx2, which are conjugated to Europium as a fluorescent tag), and three capture antibodies: (a) anti-*E. coli* monoclonal antibodies specific to Stx1 or Stx2, which are affixed at the "Stx1 Test Line" or "Stx2 Test Line" positions of the test strip, and (b) a polyclonal goat anti-mouse IgG antibody, which is affixed at the "Control Line" position of the test strip.

The Aioprep sampling device (the "Aioprep") is pre-filled with blue tinted Sample Diluent / Negative Control and contains a filter and a dropper tip. A sample of the patient's culture enriched stool specimen is transferred from the enrichment media to the Aioprep, using either the included transfer pipettes (Pipette II) or a polyester swab (not provided with the kit) to add the sample directly into the Sample Diluent / Negative Control. The diluted sample is mixed directly in the Aioprep barrel and then dispensed drop-wise into the sample port of the Curian Shiga Toxin test card.

If Stx1 and/or Stx2 antigens are present in the sample, they bind to the respective monoclonal detector antibodies conjugated to fluorescent particles, forming a complex. As the complex moves through the test strip, the anti-Shiga toxin 1 and/or 2 monoclonal capture antibodies, each bound to the assay membrane at their respective Stx1 and Stx2 test positions of the strip, bind the complexes to yield the respective test lines. When antigen is not present, a complex is not formed, and therefore, test lines do not form. As the sample continues to move further up the test strip, the polyclonal capture antibody, bound to the assay membrane at the control position of the strip, binds the conjugated antibodies and yields the control line. A line at the control position of the test strip should be present each time a sample or external control is tested. If the control line is not generated, adequate sample flow has not occurred, and the Curian Analyzer will consider the test invalid. The output is a reported test result produced by the Curian Analyzer, indicating if Stx1 and/or Stx2 has been detected. The assay detects and differentiates between Stx1 and Stx2 in a single test device.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Shiga Toxin Quik Chek

B Predicate 510(k) Number(s):
K121364

C Comparison with Predicate(s):

Device & Predicate Device(s):	**Device** K222829	**Predicate** K121364
Device Trade Name	Curian Shiga Toxin	Shiga Toxin Quik Check
General Device Characteristic Similarities		
Intended Use/Indications For Use	The Curian Shiga Toxin assay, for use with the Curian Analyzer, is a rapid, qualitative, fluorescent immunoassay for the simultaneous detection and differentiation of Shiga toxin 1 (Stx1) and	The SHIGA TOXIN QUIK CHEK test is a rapid membrane enzyme immunoassay for the simultaneous qualitative detection and differentiation of Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) in

	Shiga toxin 2 (Stx2) in a single test device. It is intended for use with cultures derived from human stool specimens to aid in the diagnosis of disease caused by Shiga toxin producing *Escherichia coli* (STEC) infections. Test results are to be used in conjunction with the patient's clinical symptoms and history.	a single test device. It is intended for use with human fecal samples from patients with gastrointestinal symptoms to aid in the diagnosis of disease caused by Shiga toxin producing *Escherichia coli* (STEC). It may be used with fecal specimens, or broth or plate cultures derived from fecal specimens. The test results should be considered in conjunction with the patient history.
Classification	Class I	Same
Product Code	GMZ	Same
Regulation	21 CFR 866.3255	Same
Measured Analyte	Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2)	Same
Antibody Format	Monoclonal/Polyclonal	Same
Target Population	Persons suspected of having disease caused by Shiga toxin producing *E. coli* (STEC) infections	Same
Type of Test	Qualitative	Same
Specimen Type	Human Stool Specimen	Same
Sample Matrix	Broth and Plate Cultures Derived from Human Stool Specimens	Same
Controls	Positive and negative control included in the kit. Internal Control line.	Same
Read Result Time	< 30 minutes	Same
Format	Single Use Cassette	Same
Storage	Refrigerated (2-8 ºC)	Same
General Device Characteristic Differences		
Sample Matrix	Not for use with Direct	Direct Human Stool

	Human Stool Specimen	Specimen
Technology	Lateral flow fluorescent immunoassay	Rapid membrane enzyme immunoassay
Interpretation of Results	Results interpretation automated by Curian Analyzer	Visual Reading

VI Standards/Guidance Documents Referenced:

- BS EN ISO 23640:2015 In vitro diagnostic medical devices - Evaluation of stability of in vitro diagnostic reagents
- ISO 14971 Third edition. 2019-12 Medical devices – Application of risk management to medical devices

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:
   Reproducibility of the Curian Shiga Toxin assay was assessed across three external testing sites. Each site conducted testing using three Curian Shiga Toxin kit lots on three independent Curian Analyzers (one per site). Testing was performed by two operators per site over the course of five days for a total of 900 data points (300 per site). Each operator was supplied with a panel of blinded specimens prior to testing. The specimen panels were tested according to the Curian Shiga Toxin package insert instructions.

   Contrived panel samples were prepared by spiking purified and quantified toxin (Stx1 and Stx2) into stool cultured negative MacConkey broth at antigen concentrations above, near, and below the assay limit of detection for Shiga toxin. Samples were prepared in bulk cultured negative MacConkey broth then diluted for consistency across all panels. For more information on the use of MacConkey broth, see section VII.B.2 Matrix Comparison. Each site received a total of 10 panels with each panel comprising 30 samples. Each panel consisted of the following in triplicate:

Table 1. Reproducibility Panel Composition

Panel Sample	Shiga Toxin Type
True Negative	No Stx 1 or Stx2
High Negative (<1xLoD)	Stx 1
High Negative (<1xLoD)	Stx 2
High Negative (<1xLoD)	Stx 1 and Stx 2
Low Positive (2xLoD)	Stx 1
Low Positive (2xLoD)	Stx 2
Low Positive (2xLoD)	Stx 1 and Stx 2
Moderate Positive (4.5xLoD)	Stx 1
Moderate Positive (4.5xLoD)	Stx 2

Moderate Positive (4.5xLoD)	Stx 1 and Stx 2

Results of the reproducibility panel are shown below in Table 2. The results are presented as % Agreement to the expected result.

Table 2. Reproducibility Panel Results

Panel Sample	% Agreement (n)	
	Stx1 Results	**Stx2 Results**
Shiga Toxin 1 Only Sample		
True Negative	100% (90/90)	100% (90/90)
High Negative	100% (90/90)	100% (90/90)
Low Positive	100% (90/90)	100% (90/90)
Moderate Positive	100% (90/90)	100% (90/90)
Shiga Toxin 2 Only Samples		
True Negative	100% (90/90)	100% (90/90)
High Negative	100% (90/90)	97.8% (88/90)
Low Positive	100% (90/90)	100% (90/90)
Moderate Positive	100% (90/90)	100% (90/90)
Shiga Toxin 1 and 2 Samples		
True Negative	100% (90/90)	100% (90/90)
High Negative	95.6% (86/90)	98.9% (89/90)
Low Positive	100% (90/90)	100% (90/90)
Moderate Positive	100% (90/90)	100% (90/90)

Positive, negative and internal controls were run on each day of testing. All positive and negative controls yielded the expected results. Reproducibility was unaffected by Curian Shiga Toxin kit lot or Curian Analyzer. These results are acceptable.

2. Linearity:

Not Applicable

3. Analytical Specificity/Interference:

Cross-Reactivity and Microbial Interference

The Curian Shiga Toxin test was evaluated for cross-reactivity and microbial interference with the bacteria and viruses listed in Tables 3 and 4 below. Each organism was spiked into a true negative cultured MacConkey broth, one contrived Stx1 positive spiked at 3x LoD into cultured MacConkey broth, and one contrived Stx2 positive spiked at 3x LoD into cultured MacConkey broth. Each preparation was tested in triplicate. Bacteria were spiked at a concentration of $\geq 1.2 \times 10^7$ CFU/ml while viruses were spiked at a concentration of $\geq 1.0 \times 10^5$ $TCID_{50}$ units per ml.

Table 3. Bacterial Strains Evaluated For Cross-Reactivity

Organism	
Areomonas hydrophila (ATCC 35654)	*Helicobacter pylori* (CCUG 39500)
Bacilius subtilis (ATCC 6051)	*Klebsiella pneumoniae* (ATCC-BAA 1900)
Bacteroides fragilis (ATCC 23745)	*Lactobacillus acidophilus* (ATCC 4356)

Campylobacter coli (ATCC 43482)	*Proteus vulgaris* (ATCC 6380)
Campylobacter concisus (ATCC 33237)	*Providencia stuartii* (ATCC 33672)
Campylobacter fetus (ATCC 25936)	*Pseudomonas aeruginosa* (ATCC 10145)
Campylobacter hyointestinalis (ATCC 35217)	*Pseudomonas fluorescens* (ATCC 13525)
Campylobacter jejuni (ATCC 33292)	Salmonella enterica susb. enterica serovar Hilversum (ATCC 15784)
Candida albicans (ATCC 18804)	Salmonella enterica susb. enterica serovar Typhimurium (ATCC 13311)
Citrobacter freundii (ATCC 43864)	Salmonella minnesota (ATCC 9700)
Clostridium difficile (ATCC 43255)	*Serratia liquefaciens* (ATCC 27592)
Clostridium perfringens (ATCC 12915)	*Serratia marcescens* (ATCC 43862)
Enterobacter cloacae (ATCC 15337)	*Shigella boydii* (ATCC 9207)
Enterococcus faecalis (ATCC 49532)	*Shigella dysenteriae* (ATCC 9361)
Escherichia coli (non-toxigenic) (ATCC BAA-2190)	*Shigella flexneri* (ATCC 12022)
Escherichia coli EIEC (ATCC 43893)	*Shigella sonnei* (ATCC 25931)
Escherichia coli EPEC (ATCC 33780)	*Staphylococcus aureus* (ATCC 51153)
Escherichia coli ETEC (ATCC 35401)	*Staphylococcus aureus* (Cowan's) (ATCC 12598)
Escherichia coli O157:H7 (non-toxigenic) (ATCC 700728)	*Staphylococcus epidermidis* (ATCC 49134)
Escherichia fergusonii (ATCC 35469)	Streptococcus equisimilis subsp. Dysgalactiae (ATCC 12388)
Escherichia hermanii (ATCC 33650)	*Yersinia enterocolitica* (ATCC 23715)
Gardnerella vaginalis (ATCC14019)	

Table 4. Viral Strains Evaluated for Cross- Reactivity

Organism	Strain ID
Human Adenovirus 2	Type 2; Species C
Human Adenovirus 14	VR-15
Human Adenovirus 40	Dugan
Human Adenovirus 41	Tak
Human Coxsackievirus A9	VR-1311
Human Coxsackievirus B1	VR-687
Human Enterovirus 69	Tolouca-1
Human Rotavirus	RV4
Feline calicivirus	VR-782

Shigella dysenteriae produces a toxin nearly identical to Stx1 and was expected to cross-react with the assay by causing a Stx1 false positive result in both the negative sample and the contrived Stx2 positive sample. *S. dysenteriae* was found to be Stx1 positive at concentrations $\geq 1.25 \times 10^6$ CFU/mL in the Curian Shiga Toxin assay. None of the other organisms tested in this study showed cross-reactivity or microbial interference in the Curian Shiga Toxin assay

Interfering Substances
The Curian Shiga Toxin test was evaluated with the substances and concentrations listed in Table 5. For this study, interfering substances were spiked at the indicated concentrations in both true negative cultured broth and contrived low positive cultured broth samples for Stx1 and Stx2 each containing Shiga toxin purified antigen at 3X LoD. None of these substances were shown to interfere with the performance of the Curian Shiga Toxin assay.

Table 5. Interfering Substance Panel

Substance (active ingredients)	Concentration in stool specimen
Barium Sulfate	5% w/v
Ciprofloxacin	0.25% w/v
Hog gastric mucin	3.5%w/v
Human blood	40%v/v
Human hemoglobin	10%w/v
Human urine	5% w/v
Imodium A-D	5%w/v
Kaopectate	5% w/v
Leukocytes	0.05%w/v
Mylanta	8.4 mg/ml
Palmitic Acid/Fecal Fat	40% w/v
Pepto-Bismol	5%w/v
Prilosec OTC	5ug/ml
Stearic Acid/Fecal Fat	40% w/v
Tagamet	5ug/ml
TUMS	50 ug/ml
Naproxen sodium	0.5% w/v
Metronidazole	0.25% w/v
Vancomycin	0.25%w/v

High-Dose Hook Effect/Prozone
A high-dose hook effect (prozone) study was conducted to evaluate potential interference from high concentrations of Shiga Toxin antigen. Contrived positive samples were prepared by diluting purified and quantified Stx1 and Stx2 in negative cultured MacConkey broth and tested in quintuplicate at concentrations ranging from 0.932 to 69.78 ng/mL for Stx1 and 0.932 to 69.78 ng/mL for Stx2. An additional sample was prepared for a contrived combined Stx1 and Stx2 positive sample representing the highest concentration of Stx1 and Stx2 tested individually (69.78 ng/mL). No false negative results suggestive of a high-does hook effect were observed.

Analyze Now Mode and Incubate and Analyze Mode/Temperature Study
The purpose of this study was to document testing results of the Curian Shiga toxin assay in Incubate and Analyze mode and Analyze Now read modes on the Curian Analyzer. In the Incubate and Analyze mode, the cassette with loaded sample was developed for 20 minutes on the bench top before insertion into the Curian Analyzer for scanning. In Analyze Now mode, the cassette was placed in the Curian Analyzer immediately and developed for 20 minutes before scanning. Additionally, testing was performed at different ambient

temperatures, including each of the following environmental conditions: <19°C, 19-27°C, and >27°C.

For this study, contrived samples and clinical specimens (both positive and negative for Shiga toxin) were evaluated. Contrived samples were generated by spiking purified Shiga toxin (Stx1 and Stx2) into negative cultured MacConkey broth at the LoD, 3x LoD and 5x LoD. A sample was tested with Stx1 and Stx2 combined at 5x LoD. All positive and negative samples were in 100% agreement with the expected results regardless of the temperature and reading mode. This study demonstrated that the correct results can be obtained with the Curian Shiga Toxin assay when the test materials are stored and tested at temperatures from 19°C to 27°C in both Analyze Now Mode and Incubate and Analyze Modes on the Curian Analyzer.

Two additional scenarios were tested to determine the effect of adding less than the recommended sample to the card (2 drops vs 3 drops) and incubating for longer than recommended time frames (up to 30 minutes vs recommended 20 minutes). The accuracy of the assay was affected by these scenarios. The labeling was updated to indicate that failure to add the recommended number of drops of prepared sample from the Aioprep onto the test card may lead to invalid test results, and over incubation of tests may lead to false-positive or invalid test results.

4. Assay Reportable Range:

   Not Applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Assay Controls

   Internal Control
   Each Curian Shiga Toxin test card contains an internal control line to confirm correct sample flow and active reagents for the test run. If the internal control line fluorescence does not meet or exceed the cut-off level, the result is considered invalid and the test should be repeated.

   External Controls
   The Curian Shiga Toxin positive and negative controls are recommended to be run with each new kit shipment or lot. One vial of positive control reagent (purified antigen) is provided with each test kit, and the kit sample diluent buffer serves as the negative control. The positive and negative controls are intended to ensure that the kit is functional to produce the expected results. The kit should not be used if either external control fails to deliver the correct test results.

   Stool Specimen Storage Stability
   Studies were conducted to examine the stability of stool samples (unpreserved or preserved in ThermoScientific Remel Cary-Blair or Para Pak C&S) when stored refrigerated (2°C to 8ºC) or frozen (-28°C to -16ºC) for defined periods of time. The contrived positive stool samples contained an Stx1 and Stx2 positive *E. coli* strain at a concentration of 6.0E+07 CFU/mL. The results demonstrated that the organisms are viable after storage for up to two

hours refrigerated (2°C to 8ºC) and up to 14 days frozen (-28°C to -16ºC). Stools preserved in ThermoScientific Remel Cary-Blair or Meridian Para Pak C&S can be stored for up to 5 days refrigerated (2°C to 8ºC) and up to 14 days frozen (-28°C to -16ºC) without impact to organism viability.

Broth Culture Storage Stability
Studies were conducted to examine the stability of broth culture enriched stool specimens when stored at three (3) different temperatures: refrigerated (2 to 8° C); -20° C (Freezer Type 2; -28 to -16° C), and -80° C (Freezer Type 1; -84 to -66° C) and defined time points. Contrived positive broth samples contained 5X LoD, 2X LoD, and < 1X LoD of Stx1 and Stx2 purified antigen. The results demonstrated that toxin can be recovered after storage up to 7 days at refrigerated temperature (2-8 C) or for up to 21 days frozen (-20°C and/or -80°C) for clinical broth culture enriched stool specimens prior to testing.

Diluted Specimen Storage Stability
Studies were conducted to examine the stability of processed broth and plate cultures in the AioPrep/Sample Dilutent stored at room temperature (19°C - 27°C) and refrigerated (2°C to 8°C) for defined time points prior to testing with the Curian Analyzer. Contrived positive cultured broth samples were prepared by diluting purified and quantified Stx1 or Stx2 into negative MacConkey broth to concentrations < 1x LoD, 2x LoD, and 5X LoD. Contrived plate cultures were prepared using one non-toxigenic strain, one Stx1 positive strain, one Stx2 positive strain, and a dual positive strain (Stx1 positive and Stx2 positive) plated to SMAC. The study results for processed broth cultures supported recommendation of storage of 8 hours or less at room temperature (19°C - 27°C) and 24 hours or less when refrigerated (2°C to 8°C). The study results for processed plate cultures supported the recommendation of storage of 2 hours or less at room temperature (19°C - 27°C) and 24 hours or less when refrigerated (2°C to 8°C).

Broth Culture Freeze/Thaw Stability
Studies were conducted to examine the stability of the broth culture enriched stool specimens when subjected to freeze/thaw cycles. Contrived positive broth samples contained 5X LoD, 2X LoD, and < 1X LoD of Stx1 and Stx2 purified antigen. The study results for processed plate cultures supported recommendation that cultured broth samples stored at -20ºC can undergo no more than two freeze/thaw cycles.

6. Detection Limit:

Limit of Detection
The limit of detection (LoD) for the Curian Shiga Toxin test was determined by spiking purified Shiga toxin (Stx1 and Stx2) into negative cultured MacConkey broth at known concentrations. Three lots of the Curian Shiga Toxin assay were evaluated in the study. The initial LoD was established by testing each of five dilutions in triplicate. The initial LoD concentrations were confirmed by testing an additional 20 replicates at the target concentrations. The LoD is the toxin concentration that yields a positive result 95% of the time and a negative result 5% of the time. The LoD for the Curian Shiga toxin test was determined to be 0.185 ng/mL for Stx1 and 0.125 ng/ml for Stx2.

Inclusivity Study
The reactivity of several clincial Shiga toxin-producing *Escherichia coli* strains (STEC) were evaluated in the Curian Shiga Toxin assay by the Sorbitol MacConkey Agar (SMAC) plate, Gram Negative (GN) broth, and MacConkey (MAC) broth culture enrichment methods. Samples were prepared by placing one Microbank bead for each strain in each media type (Gram Negative Broth, MacConkey Broth, and Sorbitol MacConkey Agar plates) and culturing at 35 - 39° C for either 16 - 24 hours (for broth enrichment method) or 18 - 24 hours (for SMAC plate enrichment method). All strains representing various serotypes and toxin combinations tested showed reactivity with the Curian Shiga Toxin assay, detailed below:

Stx1 Strains: O26:H11 (6), O111:H8 (3), O45:H2 (3), O103:H25 (2), O145 (2), O103:H11 (2), O157:H7, O103:H2, O111

Stx2 Strains: O121:H19 (4), O145 (3), O104:H21 (2), O113:H21 (2), O157:H7, O157:NM, O145:H25, O145:H28, O91:H21

Stx1+2 Strains: O111:H8 (3), O157:H7 (2), O111 (2), O145, O113:H21, O26:H11

7. Assay Cut-Off:

   To determine the preliminary cut-off the Curian Shiga Toxin assay read on the Curian Analyzer, a panel of clinical negative and contrived positive (near LoD) broth cultures were evaluated. The initial cut-off was validated during the prospective clinical study by testing a population of 1538 specimens, of which 5 were positive for Stx1, 4 were positive for Stx2, and 1529 were negative by the Curian Shiga Toxin assay. The results of the cut-off study were acceptable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Not Applicable.

2. Matrix Comparison:

An equivalency study was conducted to demonstrate that Gram Negative (GN) broth and MacConkey (MAC) broth culture enrichment methods provided comparable results. Both broth culture enrichment methods yielded positive results on the Curian Shiga Toxin assay. Results of this testing demonstrated equivalence and supports the use of MacConkey broth only in all analytical studies.

The equivalency of MacConkey broth cultures and Sorbitol MacConkey Agar (SMAC) enrichment methods was also studied. Both enrichment methods yielded positive results on the Curian Shiga Toxin assay. Results of this testing demonstrated equivalence and supports the use of MacConkey broth only in all analytical studies.

C Clinical Studies:

1. Clinical Sensitivity:

Prospectively Collected Specimens Study
The performance of the Curian Shiga Toxin test was assessed with preserved and unpreserved stool specimens prospectively collected from patients suspected of having an *E. coli* infection for whom a diagnostic Shiga Toxin test was ordered. Specimens were collected from five independent clinical sites. For the reference method, stool specimens were tested directly using the Vero cell culture method. For the Curian assay, the stool specimens were inoculated into appropriate broth and tested. The sensitivity and specificity for the Curian Shiga Toxin test was calculated by comparing to Vero cell culture performed at one central laboratory. Prospective testing consisted of 1538 stool specimens.

The ages of patients ranged from less than 2 years to over 65 years with 29.1% of the specimens coming from patients that were <21 years of age. Of the 1538 patients tested, 56.4% were female and 42.8% were male. Table 6 and 7 show a summary of the clinical performance of the Curian Shiga Toxin for all sites, combined. There was no observable difference in performance of the Curian Shiga Toxin assay with respect to study site, broth type, kit lot, or patient gender or age. These results are acceptable.

Table 6. Prospective Clinical Performance for Stx1

		Vero Cell Assay		
		Stx1 Positive	Stx1 Negative	Total
Curian Shiga Toxin	Stx1 Positive	5	9	14
	Stx1 Negative	0	1524	1524
	Total	5	1533	1538
Sensitivity		100% (5/5); 95% CI: (56.6, 100%)		
Specificity		99.4% (1524/1533); 95% CI: (98.9, 99.7%)		

Table 7. Prospective Clinical Performance for Stx2

		Vero Cell Assay		
		Stx2 Positive	Stx2 Negative	Total
Curian Shiga Toxin	Stx2 Positive	4	7	11
	Stx2 Negative	0	1527	1527
	Total	4	1534	1538
Sensitivity		100% (4/4); 95% CI: (51.0, 100%)		
Specificity		99.5% (1527/1534); 95% CI: (99.1, 99.8%)		

Archived Specimens Study
Due to low prevalence during the study period, 140 archived stool samples were retrospectively tested for Stx1 and Stx2 using the Curian Shiga Toxin assay at all five study sites. Five of the specimens were inconclusive when tested by Vero Cell Culture Assay and were removed from the study. Therefore, results for 135 archived samples were evaluated. Of the 135, 70 were negative and 65 were positive for Shiga Toxin. Tables 8 and 9 a summary of the performance of the Curian Shiga Toxin for all sites, combined, using

archived samples. There was no observable difference in performance of the Curian Shiga Toxin assay with respect to study site, broth type, kit lot, or patient gender or age. These results are acceptable.

Table 8. Prospective Clinical Performance for Stx1

		Vero Cell Assay		
		Stx1 Positive	Stx1 Negative	Total
Curian Shiga Toxin	Stx1 Positive	46	2	48
	Stx1 Negative	0	87	87
	Total	46	89	135
Sensitivity		100% (5/5); 95% CI: (92.3, 100%)		
Specificity		97.8% (1524/1533); 95% CI: (92.2, 99.4%)		

Table 8. Prospective Clinical Performance for Stx2

		Vero Cell Assay		
		Stx2 Positive	Stx2 Negative	Total
Curian Shiga Toxin	Stx2 Positive	32	2	34
	Stx2 Negative	1	100	101
	Total	33	102	135
Sensitivity		97.0% (4/4); 95% CI: (84.7, 99.5%)		
Specificity		98.0% (1527/1534); 95% CI: (93.1, 99.5%)		

These results are acceptable.

2. Clinical Specificity:
   Not applicable

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   Not applicable

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

Not applicable.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.